Original Article

# ACECLOFENAC LOADED SOLID LIPID NANOPARTICLES: OPTIMIZATION, *IN VITRO* AND EX-VIVO EVALUATION

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## ABSTRACT

**Objective:** Rheumatoid arthritis is treated with aceclofenac as the first line of treatment. Aceclofenac is a BCS-II drug, when taken orally, causes first-pass metabolism and is partially water-insoluble causing some gastrointestinal (GI) issues. Objective of the work was preparation and evaluation of solid lipid nanoparticle for rheumatoid arthritis and increases the drug permeability and further possibility to decreasing the irritation.

**Methods:** Different formulations batches were prepared by soya lecithin, tween 20, bees wax and cetyl alcohol lipid using lipid extrusion method. Further, the selected batch proceeded for Particle size, zeta potential, entrapment efficiency, *in vitro* release, Fourier transformed infrared (FTIR), X-ray diffraction studies (XRD), and ex-vivo study in goat skin.

**Results:** As a result, selected batch have nanometric in size, 72.44% entrapment efficiency, and sustained drug released (71.33%) in 6 h. The Fourier transformed-infrared studies (FT-IR) and X-ray diffraction (XRD) study confirm the compatibility of the drug with excipients. Ex-vivo permeation study showed 74.85% in drug permeate in 6 h through goat skin.

**Conclusion:** Research demonstrates that particle size and entrapment effectiveness are significantly influenced by the level of lipid and lecithin present. Additionally, it is suitable for the treatment of arthritis.

**Keywords:** Aceclofenac, Solid lipid nanoparticles, Rheumatoid arthritis, Lipid extrusion

## INTRODUCTION

Traditional formulation refers to a method of developing and manufacturing pharmaceutical products using conventional techniques (such as extraction, formulation, purification, quality control, etc.) and ingredients that have been used for many years. This approach involves blending active pharmaceutical ingredients (APIs) with excipients and other additives to create a stable and effective drug product. While traditional formulations have been used for centuries and are often viewed as a safer and more natural alternative to modern pharmaceuticals, there are also some disadvantages associated with their use [1]. To overcome the disadvantages of traditional formulations, such as poor aqueous solubility and stability, membrane permeability, drug efflux, and availability, there is increasing interest and investment in the use of lipid-based systems in drug discovery and product development. Solutions, suspensions, emulsions, microemulsions, self-emulsifying drug delivery systems, liposomes, self-micro emulsifying drug delivery systems, self-nano emulsifying drug delivery systems, dry emulsions, solid lipid microparticles, and solid lipid nanoparticles are just a few options available with these systems [2]. In 1990, Muller and Gasco were the first to investigate solid lipid nanoparticles. These submicron colloidal particles are dispersed in a solution of aqueous surfactant and consist of physiological lipids [3]. They represent a new generation of sub-micron lipid emulsions with sizes ranging from 50 to 1000 nm. They can transport both hydrophilic and lipophilic drugs. The physicochemical characterization of solid lipid nanoparticles (SLN) affects both *in vivo* and *in vitro* behaviors. The typical solid lipid nanoparticles are made up of solid lipids (at 25 °C–28 °C), emulsifiers, and a suitable solvent for both lipid and non-lipid components. Solid lipid nanoparticles (SLN) are desirable because of their potential to improve pharmaceutical performance as they offer distinctive properties like large surface area, high drug loading, and phase interaction at the interface [4]. The benefits of solid lipid nanoparticles are convenience and simple to use; simple medication termination when required; a sizable application area and drug delivery capability via a chosen site of the skin, possess the necessary qualities for self-medication. For the preparation of solid lipid nanoparticles (SLN), a variety of methods are used, including ultrasound techniques, emulsification diffusion, solvent injection, solvent diffusion,

microemulsion, hot homogenization, modified high shear homogenization, and, most recently, membrane contractor technique [5]. 0.5-1% of people from all over the world suffer from Rheumatoid Arthritis which is an autoimmune disorder [6, 7]. Rheumatoid arthritis is identified by bone and cartilage demolition in joints [8]. It is affiliated with elevated exposure to cardiovascular along with interstitial lung disorder [9, 10]). Male to female rheumatoid arthritis ratio is 1: 3 where most of the cases exist between the age of 40 and 60. Rheumatoid arthritis persists through genetic and environmental factors together like human leukocyte antigen (HLA). T cells signaling and cytokines provide an important role in rheumatoid arthritis (RA) development. Elevated pro-inflammatory cytokines level, namely nuclear factor, interleukin-1β, tumor necrosis factor-α, interleukin-6, fibroblasts, prostaglandin E2, and nitric oxide [11-15]. As the first line of treatment for rheumatoid arthritis, aceclofenac is a nonsteroidal anti-inflammatory drug. When taken orally, aceclofenac undergoes first-pass metabolism and is partially water-insoluble causing some gastrointestinal (GI) issues [16]. Solid lipid-based nanoparticles were recently developed as an alternative particulate carrier system and are gaining popularity as a novel colloidal drug carrier for intravenous applications [17-19]. When compared to polymeric nanoparticles, solid lipid nanoparticles (SLN) combine the benefits of polymeric nanoparticles and o/w fat emulsions for drug delivery and offer an alternative to current traditional carriers, particularly for the delivery of poorly water-soluble compounds for both pharmaceutical and cosmetic ingredients, as well as good tolerability [20-22].

## MATERIALS AND METHODS

Cetyl Alcohol and Soy Lecithin were bought from Central Drug House (P) Ltd. Aceclofenac was obtained as a gift sample from Panacea Biotec Limited. Tween20 was bought from Qualikems Fine Chem Pvt. Ltd. Bees wax was bought from Loba Chemie Pvt. Ltd. All additional substances were of analytical grade.

### Aceclofenac sample preparation for UV spectrophotometric method

A 100 ml volumetric flask was filled with 100 mg of pure aceclofenac drug. The drug was initially dissolved using a small amount of a 50:50 methanol and water mixture. To obtain a stock solution, make

up to the mark using the same solvent. Different concentrations, such as 1, 3, 5, and 7, were diluted from this stock solution. Then, aliquots of standard aceclofenac were made in the concentration range of 1, 3, 5, 7, and 9μg/ml, and the absorbance was measured at 203 nm against a blank reagent. Plotting absorbance vs. concentration was used to prepare the calibration [23]. The calibration curve was used to estimate the sample's concentration.

### Preparation of solid lipid nanoparticles by lipid extrusion

Aceclofenac-loaded solid lipid nanoparticles were created using a lipid extrusion technique. This technique resembled the homogenization of a blank formula. Over 5 to 10 °C above its melting point, beeswax as well as cetyl alcohol were melted. A dispersion was obtained by adding aceclofenac in melted lipid and then adding an aqueous solution of surfactant i.e., mixture of lecithin soya and tween 20. Stirrer was used

to pre-mix and pre-emulsion was formed. The pre-emulsion was introduced in Polytron Homogenizer. Generally, 15000rpm for 30 min was sufficient for homogenization whereas fig. 1. Describes SLN preparation by lipid extrusion method [24, 25]. The o/w nanoemulsion was obtained which subsequently undergoes vacuum drying. Vacuum drying is an important step in formulating solid lipid nanoparticles (SLNs) for drug delivery. It involves reducing the pressure inside a chamber to remove the solvent from the SLN dispersion. The pressure and stirring are critical parameters that affect the properties of the resulting SLNs. whereas, the pressure is maintained in the range of 50-500 mbar during the drying process. Optimization of these parameters is necessary to achieve stable and effective SLNs with desirable drug-release properties [26]. The obtained SLN powder was used for further studies and table 1. Represents formulation design for SLN by lipid extrusion.
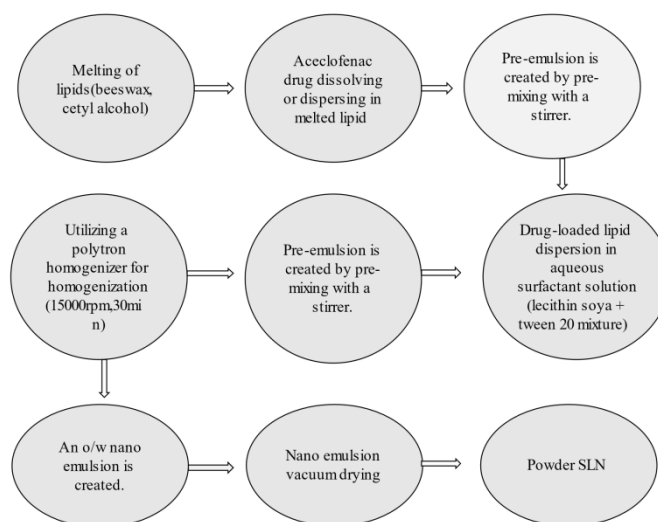


**Fig. 1: SLN preparation by lipid extrusion method**

**Table 1: Formulation design for SLN by lipid extrusion**

| Ingredients | SLN1 (mg) | SLN2 (mg) | SLN3 (mg) | SLN4 (mg) | SLN5 (mg) | SLN6 (mg) |
|---|---|---|---|---|---|---|
| Aceclofenac | 100 | 100 | 100 | 100 | 100 | 100 |
| Beeswax | 2000 | 2400 | 3200 | 4000 | 6000 | 8000 |
| Cetyl Alcohol | 1000 | 1200 | 1600 | 2400 | 3200 | 3200 |
| Soy Lecithin | 800 | 800 | 800 | 800 | 800 | 800 |
| Tween20 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| Distilled water (ml) | 200 | 200 | 200 | 200 | 200 | 200 |

### Characterization of SLNs

### Measurement of particle size and zeta potential

Using a malvern particle size analyzer and photon correlation spectroscopy (PCS), the polydispersity index (PI) and mean particle size were calculated. Disposable cuvettes made of quartz were used for the particle size and polydispersity index (PI) measurements of the SLNs using the malvern particle size analyzer and photon correlation spectroscopy (PCS) [27]. Before measurement, the samples were redispersed using handshaking after being diluted with double distilled water to an appropriate intensity of scattering [28]. The wavelength used for the measurement was 633 nm, the refractive index was set to 1.33 and a concentration of 0.1-1 mg/ml is used [29]. The measurement was performed in triplicate (n = 3).

The solid lipid nanoparticles (SLN) zeta potential was measured using a malvern zeta sizer. Disposable folded capillary cells were used for zeta potential measurement [30]. Zeta size primarily consists of lasers that are used as a light source to cast light upon the particles inside a sample. A reference beam and an incident beam are provided by this light split for determining zeta potential. This

sample cell's center got traversed with an incidental laser beam as well as the light scattered which is detected at a slant of roughly 130 degrees, is measured. Also, the wavelength and refractive index used for measuring zeta potential via the malvern zeta sizer were 633 nm and 1.33, respectively and a concentration of 0.1-1 mg/ml is used [31]. When an electric field is applied to the cell, any particles moving through the measurement volume cause the amount of light detected to fluctuate at a frequency proportional to the particles' speed. This data is then transmitted to the digital signal possessor, and finally to a computer. The frequency spectrum generated by zeta sizer software is used to calculate electrophoretic mobility and, consequently, zeta potential.

Water is commonly used as a diluent for measuring particle size, polydispersity index, and zeta potential of solid lipid nanoparticles via the malvern particle size analyzer, photon correlation spectroscopy, and malvern zeta sizer, respectively. Water is a good solvent for many drug molecules and lipid-based carriers, but its use may affect the measurements due to the solubilization of drug molecules upon dilution. The appropriate dilution ratio and time between sample preparation and measurement should be optimized

and controlled to minimize any potential effects on drug molecules' solubilisation and stability [32, 33].

### Scanning electron microscopy (SEM)

The specimen's surface morphology will be examined under a scanning electron microscope. Before mounting on brass specimens, perform double-sided adhesive tape studies. The samples are thoroughly dried in a vacuum desiccator. 120 °A alloy of gold and palladium Knees were sputtered with Argon at a plasma voltage of about 20 mA and an ambient temperature of 8 to 10 on the sample sputter coating unit (Polaron U. K. Model E5100). Sputtering started early (5 min) to ensure a uniform sample coating for scanning electron microscopy (SEM) images of high quality. The SEM was operated at a load current of about 80 mA and a low accelerating voltage of about 15 KV. The position of the condenser lens was held constant between 4.4 and 5.1. Working distance (WD) = 39 mm, and the objective lens aperture is 240 microns in size [34].

### Drug entrapment efficiency

The lipid and aqueous phases were separated by centrifuging each drug-loaded sample in a volume of 2 ml at 12500 rpm for 45 min. After dilution with methanol and filtering through 40 m filter paper, the drug content was determined using a UV-VIS spectrophotometer [35]. The SLN entrapment efficiency was calculated as

$$\% \text{ Entrapment Efficiency (EE)} = (W_a - W_s)/W_a \times 100$$

Where $W_a$: indicates the mass of aceclofenac added,

$W_s$: denotes the analyzed amount of drug in the supernatant.

### Solid lipid nanoparticles (SLN) *in vitro* release studies

*In vitro* release studies were carried out using a modified Franz diffusion cell with a 10 mg equivalent weight of solid lipid nanoparticles (SLNs) placed on the egg membrane and positioned between the donor and receptor compartments. The donor compartment is moistened using 0.5 ml of phosphate buffer. The donor compartment is filled with 50 ml of pH 7.4 phosphate buffer. A magnetic stirrer has been used to constantly stir the receptor compartment. 35 °C was kept constant. The sample was removed from the study after every 30 min, and the same volume of free phosphate buffer was substituted. The study lasted six hours [36].

### Fourier transform-infra red studies (FTIR)

Studies of the spectra of Fourier Transform-Infra Red (FT-IR) aid in identifying the drug and spotting when it interacts with polymers.

To determine the compatibility study, Fourier Transform-Infra Red (FT-IR) spectra measure for physical mixture, pure aceclofenac drug, soya lecithin, and aceclofenac loaded solid lipid nanoparticles were made [37]. Generally, a small amount of the sample is placed on a clean and transparent IR-transparent substrate such as KBr (potassium bromide) or NaCl (sodium chloride) to form a thin film. The substrate is then placed in the Fourier Transform-Infra Red (FTIR) spectrophotometer, and the infra-red (IR) beam is passed through the sample to generate a spectrum. The sample should be carefully optimized to ensure reproducible and representative spectra [38].

### X-ray diffraction studies

An X-ray diffractometer (XPERTPRO) was used to obtain an X-Ray Diffraction (XRD) pattern of drug-loaded SLN and pure aceclofenac using Ni-filtered Cu-K radiation, 45 kV, a current of 40 mA radiation, and a vertical goniometer to measure the amount of radiation dispersed in the sample's crystalline region. Diffractograms were generated at a temperature of 25 °C with a step size of 0.001 °C and a detector resolution in 2 (diffraction angle) ranging from 20 °C to 80 °C.

### Ex vivo permeability study

Goat skin was used for ex vivo studies. A hand razor was used to shave the goat skin's hair, and any adherent subcutaneous fat was carefully removed. For permeation studies, a system with a modified Franz diffusion cell was utilized. In the Franz diffusion, pre-treated goat skin was positioned so that the stratum corneum faced the donor compartment and the dermis faced the receptor compartment. 25 ml of phosphate buffer with a pH of 7.4 were placed in the receptor compartment. In the donor compartment, solid lipid nanoparticles (SLNs) weighing the same as 1 mg of aceclofenac were injected. The temperature of the diffusion medium was kept at 37 °C plus or minus 2 °C, and a magnetic stirrer was used to continuously stir the receptor compartment. For sink conditions to be maintained, samples were removed for 0, 15, 30, 45, 60, 90, and 120 min and replaced with an equal volume of fresh buffer. Samples were analysed spectrophotometrically at 203 nm after a suitable dilution.

### RESULTS

### Calibration of aceclofenac in methanol: water mixture

In a UV spectrophotometer, the absorbance of prepared test concentrations like 1,3,5,7,9 (µg/ml) is measured at a 203 nm wavelength. The regression value was found to be 0.9998 (fig. 2)
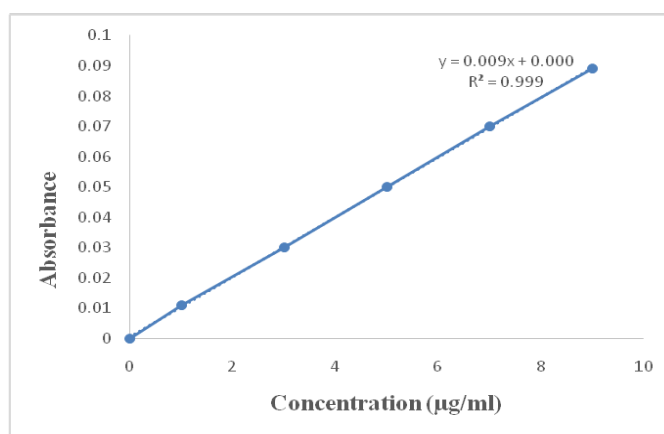


$$y = 0.009x + 0.000$$
$$R^2 = 0.999$$

**Fig. 2: Standard plot of aceclofenac in 50:50 ratio of methanol: water mixture (mean±SD)**

### Particle size and zeta potential

The mean particle size of the F6 formulation, using the Malvern particle size analyzer was 746.4±3.69 nm and the poly dispersibility of the formulation was found to be 0.377. Also, the results of the studies on the zeta potential for the F6 formulation were found to be-23.6 mV.

### Scanning electron microscopy (SEM)

Studies using scanning electron microscopy were conducted on selected formulations. Aceclofenac solid lipid nanoparticles (SLN) were smooth and nearly spherical in shape, some of the particles showed aggregation.

**Drug entrapment efficiency**

The optimized formulations F4, F5, and F6 were found to have the highest entrapment efficiency of all the formulations. And F6 has the highest level of drug entrapment in the solid lipid nanoparticles (SLN) (72.44%), which is the result of the high lipid concentration.

*In vitro* **release study**

Studies on the release of the F1, F2, F3, F4, F5, and F6 formulations were conducted *in vitro*. During the release studies, a phosphate buffer with a pH of 7.4 was used to suspend the 10 mg equivalent of the drug results shown in the table. The findings showed that for the course of the study's first 6 h, 71.33% of the drug contained in the F6 formulation was released (fig. 3 (b)).

**Fourier transform infrared studies (FTIR)**

Studies on the compatibility of the drug and excipients are measured in FTIR. FTIR spectra were obtained for the aceclofenac-loaded solid lipid nanoparticles (SLNs) and for various excipients. In the spectral range of 4000-400 cm$^{-1}$, several peaks were observed in both the aceclofenac-loaded and excipients, including an OH peak at 3298 cm$^{-1}$, C=O stretching peak at 1716 cm$^{-1}$ and CH$_2$ bending peak at 1463 cm$^{-1}$. Additionally, the aceclofenac-loaded SLNs exhibited a characteristic peak at 1739 cm$^{-1}$, assigned to the carbonyl group of aceclofenac. The drug and the other excipients did not interact, according to the Fourier Transform Infrared (FTIR) interpretation due to various excipients and the method of preparation of aceclofenac-loaded solid lipid nanoparticles. The Fourier Transform Infrared (FTIR) spectra of aceclofenac, a physical mixture, soya lecithin, and formulation are shown in fig. 4.
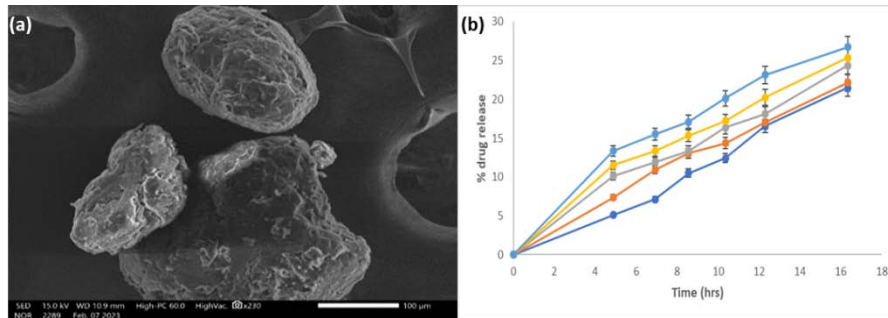


**Fig. 3: (a) The SEM image for an optimized F6 formulation, fig. scale represented the 100 µm size (b) The percentage drug release for all the SLN formulation (mean±SD, n=3)**
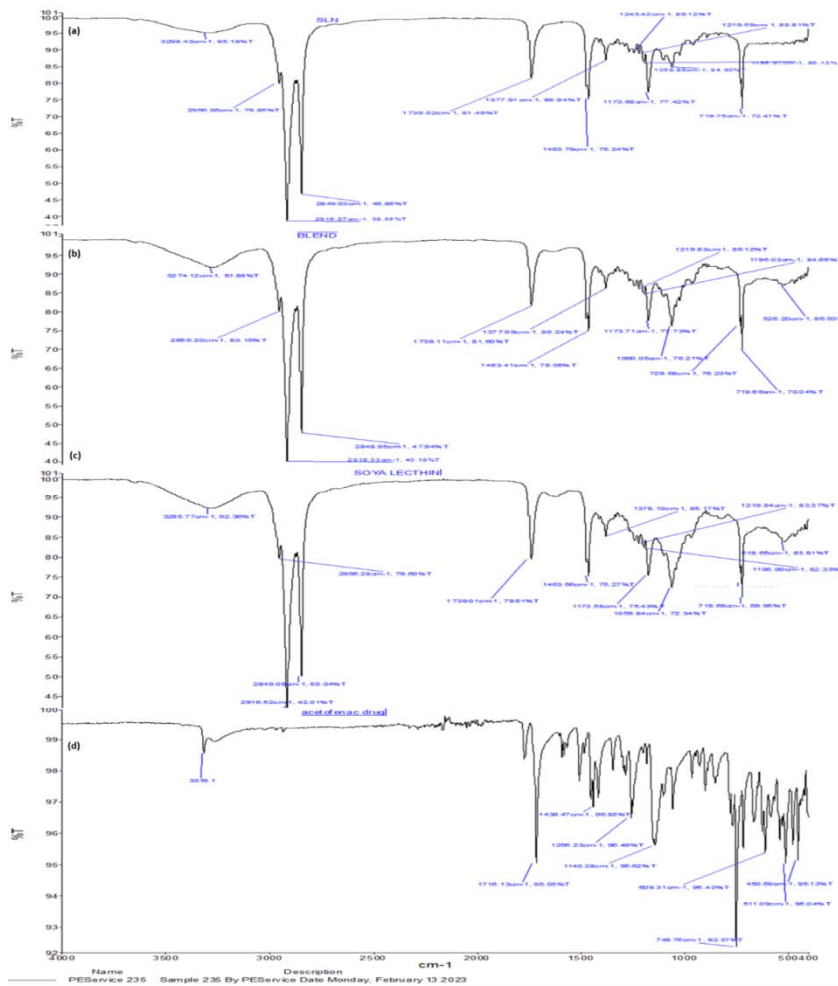


**Fig. 4: FTIR spectra of (a) SLN formulation, (b) physical mixture, (c) soya lecithin, and (d) aceclofenac**
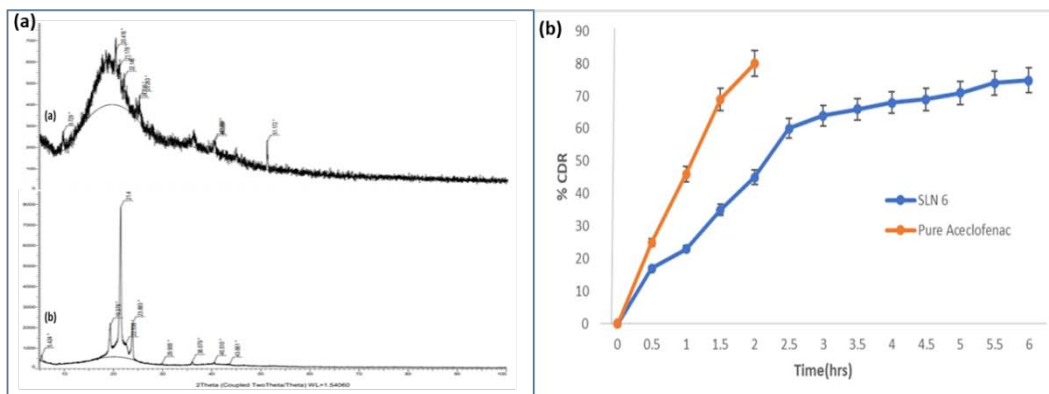
**Fig. 5: (a) X-RD graph of aceclofenac and SLN formulation (batch F6), (b) The ex-vivo percentage drug release of SLN formulation and pure drug (mean±SD)**

## X-ray diffraction studies (X-RD)

A decrease in the crystallinity of aceclofenac in solid lipid nanoparticles was indicated by XRD studies due to the high-energy processing methods, such as high-pressure homogenization, used to prepare solid lipid nanoparticles (SLNs) which can generate heat and shear forces that convert crystalline drug particles into the amorphous state. The amorphous state is characterized by a lack of long-range order and increased molecular mobility, leading to a decrease in crystallinity in XRD patterns. As a result, aceclofenac drug X-Ray Diffraction studies (X-RD) and solid lipid nanoparticles (SLN) loaded with aceclofenac were obtained (fig. 5 (a)). Aceclofenac was crystallized, as evidenced by the diffraction spectrum of pure aceclofenac.

## Ex vivo permeability studies

Fig. 7 depicts the results of ex-vivo permeation studies. In comparison to pure aceclofenac, the SLN 6 formulation has a better release rate of 74.85% in 6 h (fig. 5 (b)). However, comparing the pure drug with the aceclofenac-loaded solid lipid nanoparticle (SLN) in ex-vivo permeability studies help to evaluate the efficacy of the SLN formulation in improving drug delivery [39]. There is no interaction between drug and excipients (confirm by FTIR), thus only use a comparison of pure drug and formulation. Therefore, it is a suitable formulation for the treatment of arthritis.

## DISCUSSION

Aceclofenac-loaded solid lipid nanoparticles formulation requires a combination of aceclofenac, lipids, non-ionic surfactant, and emulsifier in specific proportions which enhance their pharmaceutical performance. The use of these components offers distinct properties, such as a large surface area, high drug loading, and phase interaction at the interface [4]. Beeswax is composed of a mixture of esters, fatty acids, and long-chain alcohols. Beeswax is commonly used due to its emollient, emulsifying, and thickening properties. Soy lecithin is a complex mixture of phospholipids, glycolipids, and other polar lipids derivatives. Soy lecithin is commonly used as an emulsifier and stabilizer. It is also used to improve the solubility and bioavailability. Tween 20 is a non-ionic surfactant composed of a polyoxyethylene sorbitan ester and a fatty acid. It is commonly used as an emulsifier, dispersant, and solubilizer. Cetyl alcohol is a fatty alcohol. It is commonly used as a thickening agent, emollient, and emulsifier due to its waxy texture and compatibility with other ingredients. The use of solid lipid nanoparticle increases the drug permeability and further lessen the possibility of irritation from entrapment. The batch is selected on the bases of particle size, entrapment efficiency, and *in vitro* release profile. Different formulations are included in the preparation using the lipid extrusion method. Further, the selected batch proceeded for morphology, Fourier transformed infrared studies (FTIR), X-ray diffraction studies (XRD), and ex-vivo study in goat skin. The optimized formulations F6 was found to have the highest entrapment efficiency of all the formulations (72.44%), which is the result of the high lipid concentration. The mean particle size of the

F6 formulation, using the Malvern particle size analyzer was 746.4±3.69 nm and the poly dispersibility of the formulation was found to be 0.377. Also, the results of the studies on the zeta potential for the F6 formulation were found to be-23.6 mV. Studies using scanning electron microscopy were conducted on improved formulations. Aceclofenac solid lipid nanoparticles (SLN) were smooth and nearly spherical. FTIR spectra were taken of the aceclofenac-loaded solid lipid nanoparticles and excipients to measure compatibility [37]. Peaks were observed in both the drug and excipients, including an OH peak at 3298 cm-1, C=O stretching peak at 1716 cm-1 and $CH_2$ bending peak at 1463 cm-1. A characteristic peak at 1739 cm-1 was seen in the aceclofenac-loaded SLNs, assigned to the carbonyl group of the drug. There was no interaction observed between the drug and excipients according to FTIR interpretation, likely due to the various excipients and method of preparation of the nanoparticles. An X-ray diffractometer (XPERTPRO) was used to obtain an X-Ray Diffraction (XRD) pattern of drug-loaded SLN and pure aceclofenac. XRD studies showed that the crystallinity of aceclofenac in solid lipid nanoparticles decreased due to the high-energy processing methods used to prepare the SLNs, such as high-pressure homogenization.

*In vitro* release studies were carried out using a modified Franz diffusion cell. Studies on the release of the F1, F2, F3, F4, F5, and F6 formulations were conducted. The findings showed that for the course of the study's first 6 h, 71.33% of the drug contained in the F6 formulation was released [36]. Goat skin was used for ex vivo studies. For permeation studies, a system with a modified Franz diffusion cell was utilized. The release rate of the SLN 6 formulation of aceclofenac was found to be better than that of pure aceclofenac, with a release rate of 74.85% in 6 h. To further evaluate the effectiveness of the SLN formulation in improving drug delivery, ex-vivo permeability studies were conducted to compare the pure drug with the aceclofenac-loaded solid lipid nanoparticle (SLN). These studies help to assess the ability of the SLN formulation to enhance drug absorption and permeability, providing valuable insights into the efficacy of the formulation for drug delivery [39, 40].

## CONCLUSION

In the lipid extrusion method, the drug-loaded solid lipid nanoparticles (SLN) were created, it has been discovered that altering the ratio of solid lipids can affect the rate of release and permeation. The solid lipid nanoparticles (SLN) were evaluated initially for their drug entrapment efficiency, zeta potential, particle size, morphology, FTIR, X-RD, and *in vitro* release profile. This research demonstrates that particle size and entrapment effectiveness are significantly influenced by the level of lipid and lecithin present. Additionally, it has been discovered that altering the ratio of solid lipids can affect the rate of release and permeation through goat skin.

## ACKNOWLEDGMENT

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